and 747 and 714 cm.$^{-1}$ for IIIa–IVa in CS_2) also support the postulated axial conformational preference for the sulfoxide oxygen. Assigning the higher frequency absorption in each pair to the equatorial C–Cl and the lower frequency absorption to axial C–Cl,[7] one finds a ratio of *axial*-chlorine to *equatorial*-chlorine absorption intensity of 0.6 for the *cis* isomer and 3.9 for the *trans* isomer. Assuming the extinction coefficient for the equatorial C–Cl stretch to be about twice that for the axial C–Cl stretch, in analogy with the results of other work[8,9] on conformational equilibria of six-ring chlorides, we conclude that there exists a large predominance of Ia for the *trans* isomer (8:1) and a very slight predominance of IIIa (*ca.* 1:1) for the *cis* isomer.

Energetically unfavorable dipole–dipole interactions have been postulated[9,10] to explain the unexpectedly large amounts of the diaxial conformers found in *trans*-1,4-disubstituted cyclohexanes when, as in the present case, both substituents are strongly electronegative. The strong parallel between the behavior of our compounds and those of Johnson which have only one electronegative substituent suggests, however, that the dipole–dipole interaction is not the only factor favoring the axial disposition of sulfoxide oxygen.

Using structural parameters determined[11] by X-ray diffraction work on an analogous sulfoxide and applying the assumptions outlined above concerning the directions of dipole moments, calculations[12] were carried out on the size of these dipole–dipole interactions for Ia, IIa, IIIa, and IVa. From these calculated values and the experimental value of ΔF, assuming[8,9] the A value for C–Cl to be 0.4 kcal., we conclude that the axial preference of the sulfoxide oxygen, in the absence of interactions with the C–Cl dipole, is real. These calculations give an A value of -0.2 to -0.6 kcal.

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Synthesis of 4-Amino-4,6-dideoxy-D-galactose and Identification with the 4-Amino-4,6-dideoxyhexose from *Escherichia coli* Strain Y-10

Sir:

Recently 4-amino-4,6-dideoxy sugars have been isolated from the antibiotic amicetin,[1] the lipopolysaccharide of *Chromobacterium violaceum*,[2] and from several strains of *E. coli* as sugars linked to thymidine diphosphate.[3,4] Amosamine, the sugar from the antibiotic, is 4,6-dideoxy-4-dimethylamino-D-glucose; viosamine, the sugar from *C. violaceum*, is 4-amino-4,6-dideoxy-D-glucose.[5] In the work reported here the 4-amino sugar isolated from *E. coli* strain Y-10 was shown to be 4-amino-4,6-dideoxy-D-galactose by comparison with a synthetic sample.

The isolation of a thymidine nucleotide from *E. coli* strain Y-10 containing an unusual acetamido sugar has been reported.[3,6,7] Oxidation of the sugar from this nucleotide with periodate resulted in uptake of two moles of periodate with formation of two moles of formic acid and one mole of an acetamido aldehyde. The latter was further oxidized with hypoiodite and then deacetylated to yield L-threonine.[8] These data established that the sugar was in the D-series and were compatible with the D-*galacto* configuration. Moreover, the sugar has been shown to be synthesized enzymatically by a pyridoxal phosphate dependent stereospecific transamination of thymidine diphosphate 4-keto-6-deoxy-D-glucose.[9]

4-Amino-4,6-dideoxy-D-galactose, its N-acetyl derivative, and crystalline tetraacetates have now been synthesized chemically and shown to be identical with the natural materials synthesized enzymatically.

Methyl 4,6-*O*-benzylidine-α-D-glucopyranoside was benzylated to give the dibenzyl derivative,[10] m.p. 97–98°, $[\alpha]^{25}D$ −27.0° (*c* 3.13, $CHCl_3$). Mild hydrolysis gave 98% yield of methyl 2,3-di-*O*-benzyl-α-D-glucopyranoside,[10] m.p. 78.5–80°, $[\alpha]^{25}D$ +17.3° (*c* 1.23, $CHCl_3$). Mesylation gave the crystalline methyl 2,3-di-*O*-benzyl-4,6-di-*O*-methylsulfonyl-α-D-glucopyranoside (I), m.p. 121–122°, $[\alpha]^{25}D$ +57° (*c* 1.14, $CHCl_3$) in 98% yield. Sodium iodide in methyl ethyl ketone at the reflux temperature selectively displaced the primary mesyl group to give 80% of methyl 2,3-di-*O*-benzyl-6-deoxy-6-iodo-4-*O*-methylsulfonyl-α-D-glucopyranoside, m.p. 133–134°, $[\alpha]^{25}D$ +41.3° (*c* 3.15, $CHCl_3$).

The primary iodo group could be smoothly reduced to the 6-deoxy derivative in 88% yield using lithium aluminum hydride in tetrahydrofuran. The resulting methyl 2,3-di-*O*-benzyl-6-deoxy-4-*O*-methylsulfonyl-α-D-glucopyranoside (II), m.p. 110–111°, $[\alpha]^{25}D$ +37.8° (*c* 2.38, $CHCl_3$) was heated with lithium azide in dimethylformamide to give in 83% yield methyl 4-azido-2,3-di-*O*-benzyl-4,6-dideoxy-α-D-galactopyranoside (III), m.p. 54°, $[\alpha]^{25}D$ +11.3° (*c* 1.94, $CHCl_3$). Compound III was reduced with lithium aluminum hydride in refluxing dioxane to methyl 4-amino-2,3-di-*O*-benzyl-4,6-dideoxy-α-D-galactopyrano-

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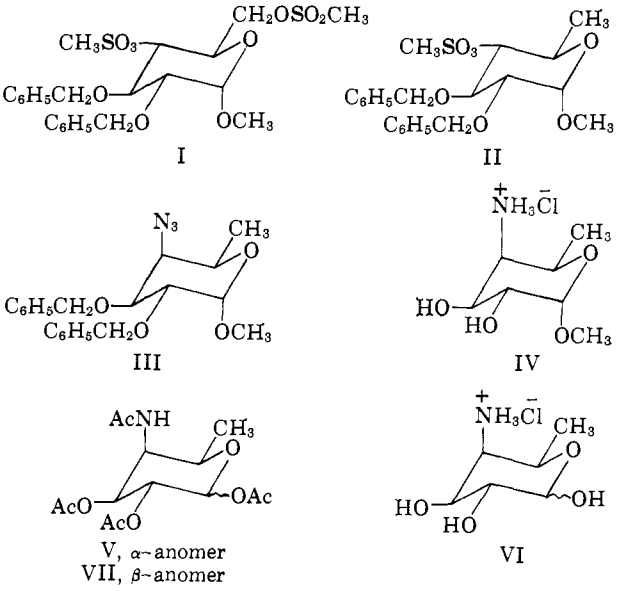
side, obtained in the form of a heavy oil in 95% yield. For characterization a small sample was converted to the hydrochloride, which melted at 199–200° and had $[\alpha]^{25}D$ +88.5° (*c* 0.89, H_2O). The benzyl ethers were cleaved by catalytic hydrogenation in the presence of two moles of hydrochloric acid and palladium on carbon as catalyst to give the crystalline hydrochloride of methyl 4-amino-4,6-dideoxy-α-D-galactopyranoside (IV), m.p. 233–234° (dec.), $[\alpha]^{25}D$ +209° (*c* 1.81, H_2O), in 76% yield. A sample of the hydrochloride IV was converted to the crystalline free base, m.p. 102–102.5°. Attempted hydrolysis of IV with dilute acid failed to give a crystalline product. Examination of the reaction mixture by paper chromatography showed the presence of some reducing material along with large quantities of the starting IV. Under vigorous hydrolysis conditions extensive decomposition occurred. In order to extend the hydrolysis studies, compound IV was converted to the N-acetyl derivative which was obtained as an amorphous solid, liquefying between 72–84°, $[\alpha]^{25}D$ +170.5° (*c* 1.86, H_2O); however, treatment of this material with 3 *N* hydrochloric acid for 1 hr. at 95–98° resulted in the selective cleavage of the N-acetyl group to give an 88% yield of IV. For an alternate hydrolysis approach, the glycoside IV was acetylated with acetic anhydride and pyridine to methyl 4-acetamido-2,3-di-*O*-acetyl-4,6-dideoxy-α-D-galactopyranoside which, without extensive purification, was treated with acetic anhydride and sulfuric acid. Chromatography of the product afforded 4-acetamido-1,2,3-tri-*O*-acetyl-4,6-dideoxy-α-D-galactopyranose (V) in 22% yield, m.p. 207–208°, $[\alpha]^{25}D$ +95° (*c* 1.1, $CHCl_3$). Hydrolysis of V with 3 *N* hydrochloric acid at 55° gave the free sugar, thomosamine, isolated as an amorphous solid. The material traveled as a single spot on paper chromatography with $R_{glucose}$ 1.13 in 1-butanol–ethanol–water, 13:8:4 (system A), and $R_{glucose}$ 2.09 in pyridine–ethyl acetate–water, 1:3.6:1.1 (system B). A small sample was converted to the N-acetyl derivative which also traveled as a single spot with $R_{rhamnose}$ 0.69 in pyridine–ethyl acetate–water, 1.4:3.6:1.15 (system C), $R_{rhamnose}$ 0.96 in system A, and $R_{rhamnose}$ 0.94 in butanol–pyridine–water, 6:4:3 (system D).

The amorphous free sugar VI was acetylated with acetic anhydride and pyridine to give 47% yield of the crystalline β-tetraacetate VII, m.p. 85–87°, $[\alpha]^{25}D$ +21.3° (*c* 1.1, $CHCl_3$).

The sugar was also synthesized with an enzyme preparation from *E. coli* strain Y-10 employing TDP-4-keto-6-deoxy-D-glucose uniformly labeled in the sugar moiety as substrate, L-glutamate as amino donor, and pyridoxal phosphate. The nucleotide product was isolated, converted to the free sugar by treatment with venom phosphodiesterase and *E. coli* phosphomonoesterase, and then the ^{14}C-4-amino-4,6-dideoxyhexose was isolated.

The following experiments have established the identity of the chemically and enzymatically synthesized compounds. Mixture of the two compounds behaved as a single, $AgNO_3$ reactive, and radioactive material in the solvent systems A and B described above. Moreover, the N-acetyl derivatives of the two compounds also behaved as single materials on paper chromatography in solvent systems A, C, and D. Finally,



a sample of the crystalline β-acetate was mixed with a solution of the radioactive 4-amino-4,6-dideoxyhexose and placed under acetylating conditions. The chemically synthesized β-tetraacetate was previously shown to be stable under these conditions. The resulting crude β-tetraacetate contained essentially all of the radioactivity and, when crystallized, had 242 counts per mg. per min. The counts remained constant through three recrystallizations[11] and thus confirmed the identity of the 4-aminosugar from *E. coli* strain Y-10 as 4-amino-4,6-dideoxy-D-galactose.

Thymidine diphosphate 4-acetamido-4,6-dideoxy-D-galactose is present in large amounts in *E. coli* strain Y-10, presumably because this rough variant of the organism is unable to synthesize some polysaccharide containing this sugar. The enzymatic mechanism for synthesis of this nucleotide has been found in one other strain of *E. coli*, in all five serotypes of *Pasteurella pseudotuberculosis*, and in seven serotypes of *Salmonella*. It can be anticipated that this sugar is a constituent of some polysaccharide in these organisms. The sugar is, however, extraordinarily labile to decomposition under the acidic conditions used for the hydrolysis of polysaccharides, which may be the reason that it has not been detected to date as a polysaccharide component despite its wide occurrence.

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(11) A small sample of this radioactive β-acetate was mixed with α-tetraacetate and shown to be removed by recrystallization.

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